Misreading of RNA Codewords Induced by Aminoglycoside Antibiotics

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SUMMARY

The aminoglycoside antibiotics streptomycin, neomycin, kanamycin, paromomycin, gentamicin, hygromycin B, and viomycin all disturb the fidelity of the reading of polyribonucleotides in polypeptide synthesis. This effect is seen with all four homopolymers: poly U, poly C, poly A, and poly I.

The extent and the spectrum of misreading vary with the antibiotic used and with the sRNA concentration. The antibiotics also influence the incorporation of the "correct" amino acid: with poly A they cause either stimulation or inhibition of lysine incorporation, depending on the sRNA concentration; poly C-directed proline incorporation was stimulated at all sRNA concentrations tested; and with poly U-directed phenylalanine incorporation the drugs were only inhibitory with most extracts. In all systems, with increasing sRNA the inhibition by the drug of "correct" reading became more prominent, and the relative misreading less prominent.

In the light of recent knowledge of coding triplets, the effects observed suggest that aminoglycosides can (a) cause misreading of only one base at a time in UUU and CCC triplets, but of all three in AAA or III; (b) cause misreading of a base in only the 5′ and the internal position in the pyrimidine codons, but in all three positions in the purine codons; and (c) allow both transition misreadings (purine to purine, or pyrimidine to pyrimidine) and transversion misreadings (purine to pyrimidine, or pyrimidine to purine). Transversion misreadings would seem to be rare in pyrimidine-containing polymers.

INTRODUCTION

Finding that streptomycin (Sm)[1] can cause phenotypic suppression of auxotrophic mutations—in both Sm-resistant (1) and Sm-sensitive (2) strains of *Escherichia coli*, Gorini and Kataja (1) concluded that the drug interferes with accurate translation of the RNA code into protein. This inference was confirmed *in vitro*, with polynucleotide-directed polypeptide synthesis

[1] The following abbreviations are used: poly U, polyuridylic acid; poly A, polyadenylic acid; poly C, polycytidylic acid; poly I, polyinosinic acid; poly UG, copolymer of uridylic and guanylic acids; poly UC, copolymer of uridylic and cytidylic acids; poly UA, copolymer of uridylic and adenylic acids (ratios given are input ratios in enzymic synthesis of copolymers); sRNA, transfer RNA; TCA, trichloroacetic acid; Sm, streptomycin; dihydroSm, dihydrostreptomycin; Blm, bluensomycin; Km, kanamycin; Nm, neomycin B; Gm, gentamicin; Vm, viomycin; Hm, hygromycin B; Pm, paromomycin.

on ribosomes from Sm-sensitive cells (3). With ribosomes from Sm-resistant cells misreading could not be detected, although it must occur (at a low level) in the cells in order to account for phenotypic suppression by the drug.

Other aminoglycoside antibiotics [neomycin B (Nm) and kanamycin (Km)] also caused misreading *in vitro*, but in somewhat different patterns from that induced by Sm (3). In the present work we have further studied the effect of these and several additional antibiotics on polypeptide synthesis, directed by various polyribonucleotides, in order to define the nature of the misreading more closely by relating the induced coding changes to the presently known codons. In the course of this work, changes in sRNA concentration were found to affect markedly both the stimulation of misreading and the inhibition of "correct" reading of polynucleotides; the drugs even stimulated the "correct" reading under certain circumstances.

Recently Pestka, Marshall, and Nirenberg (4), studying the influence of Sm on the binding of aminoacyl-sRNA to ribosomes, reached conclusions concerning the pattern of misreading, and concerning the effect of sRNA concentration, consistent with those presented here.

METHODS

Cells of *E. coli B* were harvested in log phase (about 7×10^8 cells/ml; 0.75 g wet weight per liter) from cultures grown in minimal medium supplemented with 0.2% Casamino acids (Difco), or in tryptic digest broth. Dialyzed, preincubated crude extracts (is-30) were prepared as described previously (5). The extracts were frozen in small portions in acetone-dry ice and stored at −70°. Extracts from Sm-sensitive strains were used; the concentration was determined with the assumption that 60 μg ribosomes per millilter gives an OD_{260} of 1.

Polynucleotide-directed polypeptide synthesis was carried out according to Nirenberg (5), except that NH_4Cl was used in place of KCl; 0.25-ml incubation volumes were used (except where stated), containing 0.015 ml of is-30 extracts. [The RNA content of this addition corresponded to about 150–200 μg ribosomes and 20–30 μg sRNA, assuming that rRNA:sRNA equals four (6).] The Mg^{++} concentration was 12.5 mM; poly U was used at a final concentration of 10 μg, poly C and poly A at 15–20 μg, and poly I at 40 μg per 0.25 ml. Unless otherwise stated, incubations with poly U, poly C, and poly I were supplemented with 50 μg sRNA; incubations with poly A were supplemented with 200 μg sRNA. Each incubation mixture contained one ^{14}C-amino acid plus all nineteen other ^{12}C-amino acids.

Incubations were carried out at 35° for 30 min unless otherwise stated. With poly U, poly I, and poly C, reactions were stopped by adding 1 ml of 10% trichloroacetic acid (TCA). With poly A reactions were terminated by the addition of 3 ml of a mixture of equal parts of 0.5% sodium tungstate and 10% TCA, each adjusted to pH 2.0. After heating at 90° for 15 min (the tungstate-TCA was readjusted to pH 2.0 with 10% TCA after heating), the precipitates were collected on Millipore filters, washed three times with 5% TCA, dried, and counted in a gas-flow end-window counter. All samples counted contained at least 50 times the background count. All experiments were repeated at least once; only amino acid incorporation $\geqslant 1$ μμmole per incubation mixture is recorded. Glutamine and asparagine were not tested.

MATERIALS

Polyribonucleotides (except poly I) were synthesized with polynucleotide phosphorylase from *Micrococcus lysodeikticus*, by a modification of the method of Steiner and Beers (7), from trilithium salts of UDP, CDP, ADP, and GDP, obtained from Schwarz Bioresearch. *Escherichia coli* sRNA (stripped) was obtained from General Biochemicals, and polyinosinic acid from Miles Chemical Company. Uniformly labeled ^{14}C-amino acids were obtained from Schwarz Bioresearch and from New Eng-

TABLE I

Aminoglycoside antibiotics

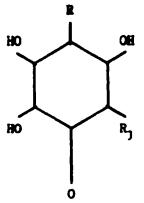
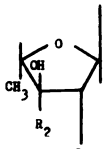
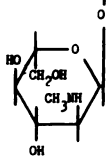
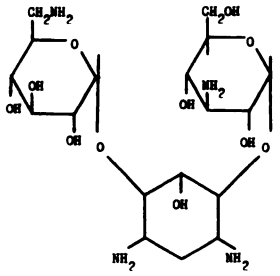
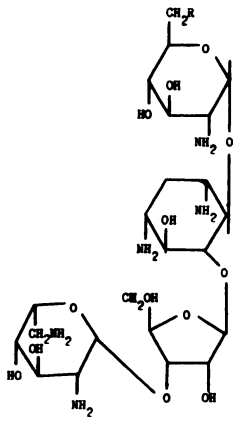
Antibiotic	Structure
Streptomycin R = R_1 = -NHC($=NH$)NH_2; R_2 = -CHO	
Dihydrostreptomycin R = R_1 = -NHC($=NH$)NH_2; R_2 = $-CH_2OH$	
Bluensomycin R_2 = $-CH_2OH$ and R = -NHC($=NH$)NH_2, R^1 = $-OCONH_2$; or R = $-OCONH_2$, R_1 = -NHC($=NH$)NH_2	
Kanamycin	
Hygromycin B	$C_{15}H_{30}N_2O_{10}$
Viomycin	$C_{18}H_{31-33}N_9O_8$
Gentamicin	$C_{17-18}H_{34-36}N_4O_7$

TABLE I (Continued)

Antibiotic	Structure
Neomycin B, C (R = NH_2)	
Paromomycin (R = OH)	

land Nuclear Corporation. Streptomycin sulfate, dihydrostreptomycin sulfate, viomycin, and paromomycin were commercial preparations. Kanamycin sulfate was provided by Dr. Joseph Lein of Bristol Laboratories, hygromycin B by Dr. R. L. Mann of Lilly Research Laboratories, gentamicin by Dr. M. J. Weinstein of the Schering Corporation; and neomycins B and C, and bluensomycin by Dr. W. T. Sokolski of the Upjohn Company.

Structure of aminoglycoside antibiotics. There are a considerable number of aminoglycoside antibiotics, some half-dozen of which are in clinical use. They appear to have closely related or identical modes of action (8–10), and this conclusion is confirmed by our findings on their ability to cause misreading in the *in vitro* incorporation system. The antibiotics used are listed in Table 1, together with their structures; at present little is known of the structures of viomycin, hygromycin B, or gentamicin, although it has been established that they contain at least one amino sugar. Viomycin does not appear to be as closely related to Sm as the other drugs. These antibiotics are all strongly basic compounds, containing multiple amino groups and/or guanidino groups.

RESULTS

EFFECT OF AMINOGLYCOSIDES ON POLYNUCLEOTIDE CODING

Polyuridylic Acid

Spectrum of misreading. Poly U is known to direct the incorporation of phenylalanine and leucine under the usual conditions of polypeptide synthesis (11). In our experiments small amounts of isoleucine, serine, and tyrosine were often also incorporated in control experiments without any drug. Their incorporation is known to be greatly increased by changes involving Mg^{++} or polyamine concentration (3, 12, 13), pH (14), temperature (12, 13), or the addition of organic solvents (15).

Table 2 shows that all the aminoglycoside antibiotics tested disturbed this system, inhibiting phenylalanine incorporation under the usual conditions. (Exceptions, under other conditions, will be discussed below.) In addition, all except viomycin (Vm) increased the incorporation of various amino acids not usually coded for by poly U. Different antibiotics exhibited different patterns of misreading: Sm gave leucine, isoleucine, and tyrosine; Nm gave less leucine than Sm, similar amounts of

TABLE 2
Amino acid incorporation with poly U in the presence of aminoglycosides[a]

Amino acid	Antibiotics,[b] 4 μg/ml							
	None	Sm	Km	Nm	Hm	Gm	Vm	Pm
	Relative incorporation[c]							
Phenylalanine	100[d]	60	70	40	50	55	22	49
Leucine	5	10	6	6	7	15	—[e]	9
Isoleucine	8	30	15	37	2	55	—	25
Serine	4	20	31	48	56	100	—	X[f]
Tyrosine	26	38	34	290	69	133	—	X

[a] Conditions as described under Methods. Data compiled from a number of experiments; 0.25 ml incubations supplemented with sRNA.

[b] Antibiotics were used at a final concentration of 4 μg/ml. This gives a concentration range of 6.5 μM for Sm to 10 μM for Gm.

[c] Results are expressed as percentage relative to net phenylalanine incorporation (corrected for incorporation in absence of poly U), without drug.

[d] Incorporation of phenylalanine (100) was 500 μμmoles per 0.25 ml incubation.

[e] Dash: <1 μμmole.

[f] X: not tested.

isoleucine, more serine, and much more tyrosine; hygromycin (Hm) gave mostly serine and tyrosine.

The results obtained with certain other antibiotics are not shown here. Neomycin C, a stereoisomer of Nm, exhibited a misreading spectrum similar to that of Nm. DihydroSm and bluensomycin (Blm) closely resemble Sm in action as well as in structure.

Sequence of addition. It has been previously reported (16, 17) that inhibition of poly U-directed phenylalanine incorporation requires that Sm be added prior to the messenger, and this requirement has been considered important for understanding the mechanism of action of the drug. In all the experiments reported here the incubation mixtures were made up in this way. However, in numerous experiments we have been unable to find any significant dependence of either inhibition or misreading on order of addition. The same conclusion has been reached by van Knippenberg *et al.* (18).

Copolymers containing U. With different mixed polymers containing U, the aminoglycosides caused either inhibition or stimulation of phenylalanine incorporation under the usual conditions. With a copolymer with a high proportion of U [UC (3:1), or UG (3:1)], just as previously observed with poly U, the aminoglycoside antibiotics were inhibitory. With a lower proportion of U [UC (1:3), or UA (1:2)], however, the drugs (except for Vm and Hm) stimulated phenylalanine incorporation with the same extracts. At a sufficiently low ratio of UUU triplets to other triplets, the misreading of other codons to give phenylalanine apparently outweighs the inhibition of the "correct" reading of UUU.

Polycytidylic Acid

With poly C, which directs the synthesis of polyproline (19), most of the aminoglycosides caused varied and marked misreading (Table 3). In contrast, however, to the inhibition of the "correct" incorporation seen with poly U, several of the drugs greatly stimulated proline incorporation with poly C under the usual conditions. Hm had only a weak stimulatory effect, and Vm was usually inhibitory.

TABLE 3
Amino acid incorporation with poly C in the presence of aminoglycosides[a]

Amino acid	Antibiotic, 4 μg/ml						
	None	Sm	Km	Nm	Hm	Gm	Pm
	Relative incorporation						
Proline	100[b]	230	260	485	130	375	390
Histidine	1	12	225	196	76	41	X
Threonine	17	41	30	171	9	76	X
Leucine	1	3	3	185	15	74	X
Alanine	1	4	2	5	—	2	X
Serine	—	55	32	200	35	67	X

[a] Conditions as described under Methods; results expressed as noted in Table 2.

[b] Incorporation of proline (100) was 200 μμmoles per 0.25 ml incubation.

Polyadenylic Acid

Poly A directs the synthesis of only polylysine *in vitro* (20) and requires more

sRNA than the other homopolymers. The aminoglycoside antibiotics tested, with the exception of Hm and Vm,[2] all caused misreading of poly A (Table 4), but to a much

TABLE 4
Amino acid incorporation with poly A in the presence of aminoglycosides[a]

Amino acid	Antibiotic, 4 μg/ml							
	None	Sm	Km	Nm	Hm	Gm	Vm	Pm
	Relative incorporation							
Lysine	100[b]	300	184	350	50	140	30	300
Valine	—	5	2	16	X	X	X	X
Arginine	—	2	1	7	X	X	X	X
Aspartic acid	—	1	—	7	X	X	X	X
Glutamic acid	—	—	—	1	X	X	X	X
Glycine	—	1	—	3	X	X	X	X

[a] Conditions as described under Methods; results expressed as noted in Table 2.

[b] Incorporation of lysine (100) was 250 μμmoles per 0.25 ml incubation.

lesser degree than that observed with poly U and poly C. The drugs that caused misreading also stimulated the incorporation of the "correct" amino acid, lysine, under the usual conditions. Inhibition was observed under other conditions, however, as will be noted below.

Polyinosinic Acid

Polyguanylic acid is not readily obtainable, but since copolymers containing I or G show identical coding properties (21), poly I would be expected to code like poly G. Poly I has been reported to be inactive as a messenger (22), perhaps because of its triple-stranded structure (23). We found, however, that poly I did stimulate the incorporation of small amounts of glycine. When aminoglycosides were added, substantial amounts of glycine and several other amino acids were incorporated (Table 5). This stimulation is unlikely to result from derangement of the triple strand of poly I by the drug, as it was not observed with Sm-resistant ribosomes.

[2] This inactivity of Hm and Vm was demonstrated by measurements of total misreading, employing ^{14}C-labeled algal hydrolyzate plus an excess of ^{12}C-lysine (unpublished).

TABLE 5
Amino acid incorporation with poly I in the presence of aminoglycosides[a]

Amino acid	Antibiotic, 4 μg/ml			
	None	Sm	Km	Nm
	Amount incorporated[b]			
Glycine	17	180	235	175
Arginine	—	125	—	30
Tyrosine	—	40	55	175
Valine	—	1010	390	364
Serine	—	—	5	18
Lysine	—	20	20	26

[a] Conditions as described under Methods.

[b] Results are expressed as μμmoles of amino acid incorporated per 0.25 ml incubation.

EFFECT OF sRNA CONCENTRATION

Poly U. The above experiments on specific misreading were done with extracts supplemented with sRNA; when this supplement was omitted most extracts showed little difference in pattern, Sm still inhibiting phenylalanine incorporation with poly U.[3]

The level of Sm-induced isoleucine incorporation was relatively high, and added sRNA caused little change in the *absolute* level of misreading (except occasionally a small decrease). The same additions, however, stimulated phenylalanine incorporation with or without the drug (Fig. A and B). Increasing sRNA concentrations therefore decreased the *relative* misreading (i.e., isoleucine compared with phenylalanine), as shown in Fig. 1C.

Other polynucleotides. The effect of aminoglycosides on poly A-directed polylysine synthesis was also strikingly dependent on sRNA concentration and ranged from stimulation to inhibition. Fig-

[3] With a few extracts, Sm was found to stimulate rather than to inhibit poly U-directed phenylalanine incorporation. Extracts showing this effect showed only a weak response to poly U, which could be improved markedly by the addition of sRNA. This addition also restored the usual inhibitory effect of Sm. The abnormal behavior of these extracts can be explained in part by assuming a lower sRNA content than usual. Studies with more complete control of sRNA concentration are in progress.

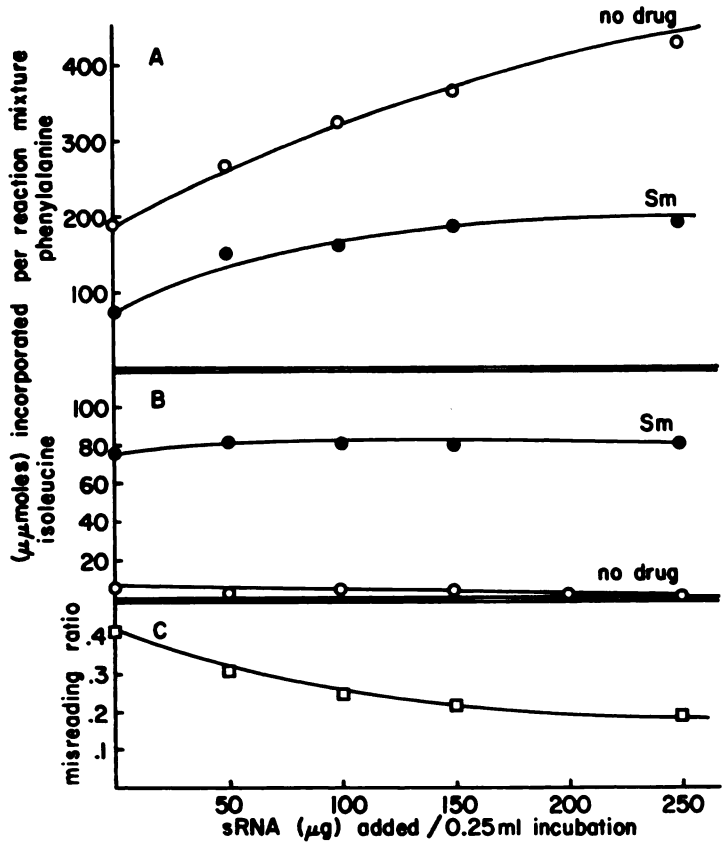


FIG. 1. *The effect of sRNA on poly U-directed incorporation (0.25 ml incubation)*
Sm at 4 μg/ml.
(A) Phenylalanine incorporation.
(B) Isoleucine incorporation.
(C) The effect of sRNA on misreading ratio (isoleucine incorporated in presence of drug): (phenylalanine incorporated in absence of drug).

ure 2 shows that the incorporation of lysine was stimulated by Sm with an unsupplemented extract, as previously noted, but was inhibited at high concentrations of sRNA.

With poly C, added sRNA itself inhibited proline incorporation at concentrations above 300 μg per 0.25 ml incubation (Fig. 3); hence only a relatively narrow range of sRNA concentration was available for testing. Within this range increasing sRNA caused an increase and then a decrease in the stimulatory effect of Nm on proline incorporation (Fig. 3). Similar effects were seen with other aminoglycosides.

Kinetics of stimulation of "correct" amino acid incorporation. These kinetics were studied to see whether the stimulation of "correct" incorporation, at the usual sRNA concentration, was due to extension of the duration of synthesis or to an increase in its rate. Fig. 4 shows that with poly A aminoglycosides allowed synthesis of polylysine for a longer period of time without perceptibly increasing its rate. However, with polyproline synthesis, directed by poly C, Nm markedly increased both rate and duration (Fig. 5).

DISCUSSION

There is strong evidence that the aminoglycoside antibiotics act on the 30 *S* ribo-

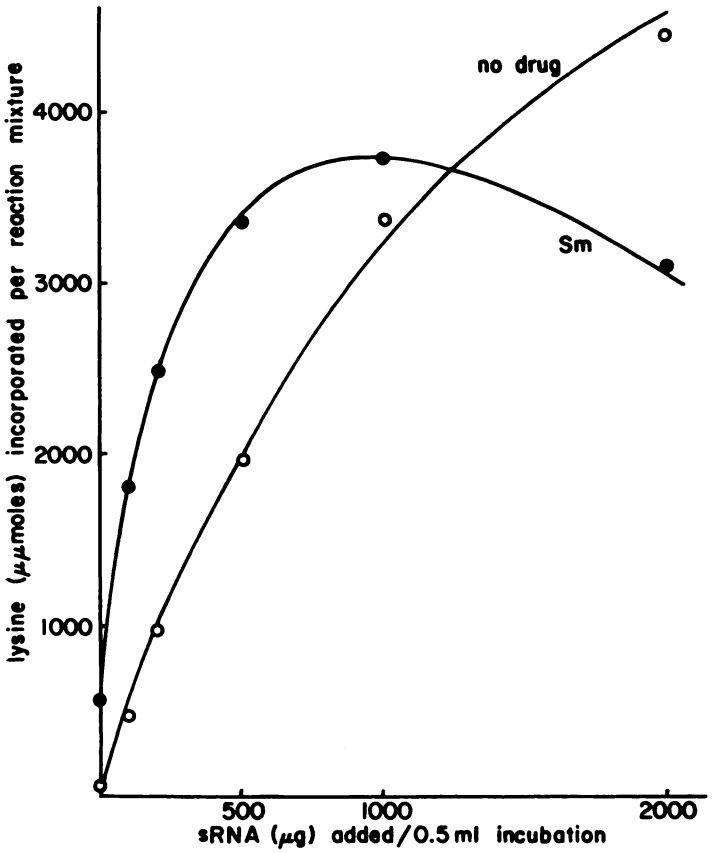


FIG. 2. *The effect of sRNA concentration on poly A-directed lysine incorporation (0.5 ml incubation)* Sm at 5 μg/ml.

some subunit (17, 24), and this is presumably the site at which they disturb the reading of the code (3). Misreading can also be promoted *in vitro* by changes in cation concentration (3, 12, 13), pH (14), or temperature (12, 13) or by addition of organic solvents (15), but it is not known whether these factors act on the 30 *S* subunit or on another component of the system; an effect of organic solvents on sRNA has been observed (25). It should be noted that aminoglycosides can produce several times as much misreading as can increased Mg^{++} or spermidine concentrations. The two effects are, in fact, additive (unpublished results).

It is known that the misreading caused by aminoglycoside antibiotics is not random: they cause poly U to code for only a few additional amino acids. The present study was carried out in an attempt to define the basis for these specific misreadings. A simple pattern, if found, might shed light on the way in which the antibiotics cause misreading, and on the mechanism of reading of the RNA code.

Specificity of misreading. The amino acid incorporations directed by each homopolymer in the presence of an aminoglycoside are listed in Table 6 together with their known codons. If we seek the minimal substitutions required, we see that the misreading of poly U can be entirely accounted for in terms of triplets with the following characteristic: (a) one base is misread; (b) that base is in the 5′-terminal or the internal position of the triplet; and (c) it is misread as though U were either C (leucine, serine) or A (isoleucine, tyrosine). However, leucine (UUA,

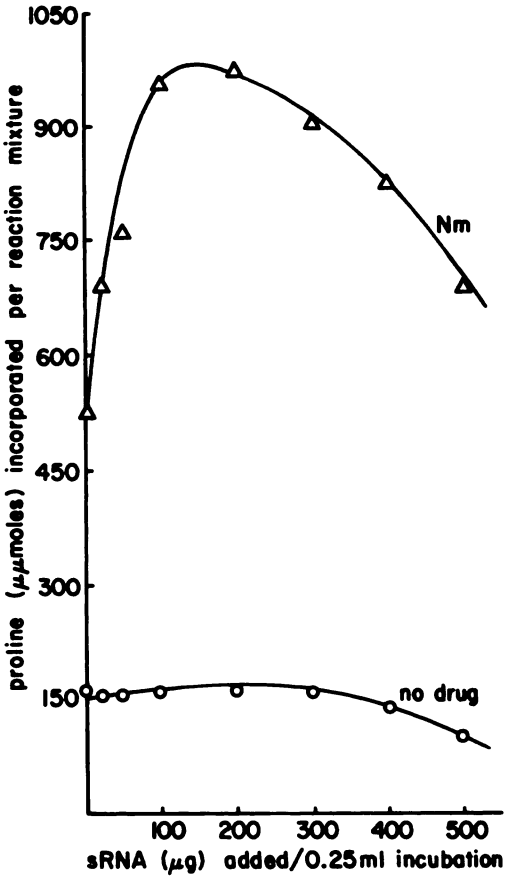


FIG. 3. *The effect of sRNA concentration on poly C-directed proline incorporation (0.25 ml incubation)*

Nm at 4 μg/ml.

UUG) could alternatively be derived via another single substitution, that of A or G in the 3′-terminal position.

The results obtained with poly C can similarly be accounted for in terms of misreading a single base in the 5′ or the internal position. C would be misread as A (histidine, threonine), U (leucine, serine), or G (alanine).

The results obtained with poly A cannot be interpreted so simply. Most of the amino acids incorporated would appear to involve two base misreadings in the triplet, and the 3′ position would appear to be involved in aspartate and glutamate. The most striking aspect of this pattern is the frequent appearance of G: every amino acid would have to involve at least one misreading of A as G; and, indeed, except for valine, and possibly aspartate, it is not necessary to postulate any misreading of A as a pyrimidine. It should be emphasized, however, that the level of misreading observed with poly A is at least 10-fold lower than that observed with either pyrimidine polynucleotide.

The use of poly I as a model for poly G has certain theoretical objections. Poly I forms a triple-stranded structure at neutral pH (23); and although I is thought to pair like G in coding, it can form only two hydrogen bonds rather than three, which may allow more flexibility in pairing. These differences between G and I may be especially important under circumstances that promote errors in reading. Indeed, the

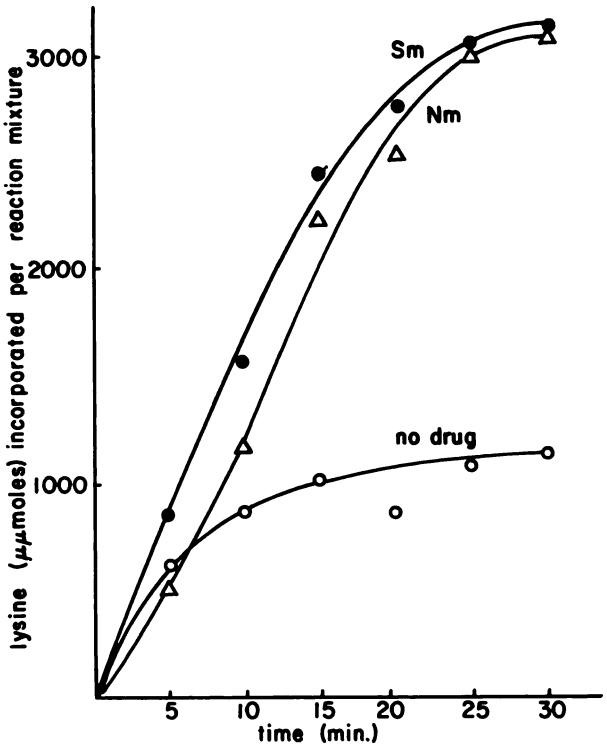


FIG. 4. *Kinetics of poly A-directed polylysine synthesis*
Samples (0.3 ml) were removed from a 2.5-ml incubation containing 0.25 ml is-30, 1.25 mg sRNA, and 150 μg poly A. Drugs at 20 μg/ml.

amino acids incorporated with poly I plus aminoglycosides have codons in which G is strikingly sparse. If we assume that I = G in coding, only glycine can be accounted for in terms of a codon containing 2 G's; the known codons for lysine, arginine, valine, and serine contain at most one G; and those for tyrosine contain none. Thus with poly I two or three of the bases of the triplet are frequently misread; but as noted above, this polymer may well not be an adequate model for studying the misreading of G.

With the pyrimidine homopolymers the aminoglycoside-induced misreading can be entirely accounted for in terms of connected triplets (26, 27), i.e., those differing from the supplied triplet by only one base (connected triplets are shown in boldface in Table 6). The degree of connectedness is even greater if U and C are considered interchangeable at the 3′-terminal position, as has been suggested by Bernfield and Nirenberg (28): such a possible misreading can be seen for *every* amino acid misread with poly U or poly C (italics in Table 6). Furthermore, it is not necessary to invoke any misreading of the 3′-position. It therefore seems possible that aminoglycosides distort the configuration of the ribosome in a way that affects the reading of the 5′- and the internal, but not the 3′-position.

With the purine homopolymers, however, no simple pattern of base misreading is apparent. It seems that aminoglycosides interfere less extensively with the reading of purines than with that of pyrimidines, since with poly A the level of misreading was low compared with poly U and poly C; the level of misreading of G, as noted above, cannot be reliably predicted.

Pestka *et al.* (4), studying the binding of aminoacyl-sRNA with nucleotide triplets on ribosomes, have found that Sm can

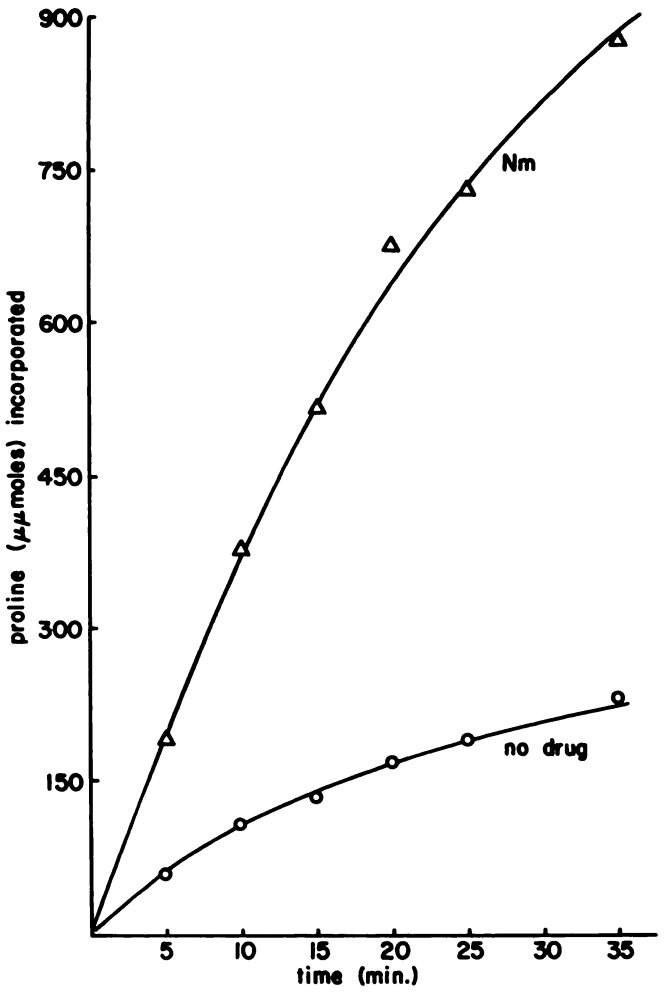


FIG. 5. *Kinetics of poly C-directed polyproline synthesis*

Samples (0.2 ml) were removed from a 2.5 ml incubation containing 0.15 ml is-30, 250 μg sRNA, and 200 μg poly C. Nm at 2 μg/ml.

alter the recognition of both internal and terminal bases.

In the misreading of the 5'- and the internal positions, not only do "transitions" occur (reading of one pyrimidine for the other), but also "transversions" (reading of A for U or C, and in one case reading of G for C).[4] Nevertheless, the aminoglycosides do not cause all possible connected codon readings. Thus, with poly U one does not see incorporation of cysteine (UGU, UGC) or valine (GUU, GUC); and with poly C one sees alanine (GCC, GCU), but not arginine (CGC, CGU). This would suggest that aminoglycosides only rarely effect the pyrimidine to G transversion.

The somewhat specific patterns of misreading induced by various aminoglycosides correlate with a recently observed specificity of suppression: certain auxotrophic mutants of *E. coli* are suppressed by Nm but not Km, and others by Km but not Nm (2). In this connection a mutant specifically corrected by Hm would be of

[4] "Transition misreadings" and "transversion misreadings" are analogous to the corresponding mutagenic conversions, but no actual replacement of bases is implied.

TABLE 6
Amino acid incorporation influenced by aminoglycosides[a]

Homopolymer codon	Amino acid incorporated	Level of incorporation[b]	Codons
UUU	Phenylalanine	H	UUU, UUC
	Isoleucine	I	**AUU**, *AUC*
	Serine	I-H	**UCU**, *UCC*, UCA, UCG, AGU, AGC
	Tyrosine	I-H	**UAU**, *UAC*
	Leucine	L	**CUU**, *CUC*, CUA, CUG, **UUA**, **UUG**
CCC	Proline	H	CCU, CCC, CCA, CCG
	Histidine	H	*CAU*, **CAC**
	Serine	H	*UCU*, **UCC**, UCA, UCG, AGU, AGC
	Threonine	I	*ACU*, **ACC**, ACA, ACG
	Leucine	I-H	*CUU*, **CUC**, CUA, CUG, UUA, UUG
	Alanine	L	*GCU*, **GCC**, GCA, GCG
AAA	Lysine	H	AAA, AAG
	Valine	L	GUU, GUC, GUA, GUG
	Arginine	L	CGU, CGC, CGA, CGG, **AGA**
	Aspartic acid, glutamic acid	L	GAU, GAC, **GAA**, GAG
	Glycine	L	GGU, GGC, GGA, GGG
III	Glycine	H	GGU, GGC, GGA, GGG
	Valine	H	GUU, GUC, GUA, GUG
	Tyrosine	H	UAU, UAC
	Arginine	I	CGU, CGC, CGA, CGG
	Lysine	L	AAA, AAG
	Serine	L	UCU, UCC, UCA, UCG, AGU, AGC

[a] These data were taken from Trupin *et al.* (29), with the exception of the AGA triplet for arginine, which has been experimentally determined by H. G. Khorana (personal communication). Sequence in codons is 5′-terminal, internal, and 3′-terminal. Codons in boldface type are connected to input codons; those in italics are connected with U and C interchanged in the 3′-terminal position.

[b] H, large effect of drug on incorporation; I, intermediate effect; and L, small effect.

particular interest, as this drug has been found to alter only the internal U of a triplet, misreading poly U to give tyrosine (UAU) or serine (UCU); the misreading with poly C is very small. To date, however, we have not been able to isolate such a strain.

A large fraction of auxotrophic mutants are not detectably suppressed by Sm, even in strains whose ribosomes do permit suppression of other auxotrophic mutations.[5] Yet both classes of mutants presumably arise by point mutation (in which a codon is converted to a connected codon). This selectivity of suppression *in vivo* is therefore in harmony with the present finding, that aminoglycoside-induced misreading *in vitro* is limited to only some members of the total set of connected triplets.

The effects of sRNA concentration. With poly A, Sm has been reported both to inhibit (18) and to stimulate[6] lysine incorporation. In the present work the aminoglycosides were seen to stimulate this incorporation at the sRNA concentrations ordinarily used *in vitro*, but to inhibit it at higher sRNA concentrations. With poly C the drugs also stimulated the "correct" incorporation (proline), but the possible similar onset of inhibition at high sRNA concentrations could not be tested because the addition of sRNA above 300 μg per incubation was itself inhibitory. With poly U, while the drugs exerted only an inhibitory effect on phenylalanine incorporation

[5] L. Gorini and E. Kataja, unpublished.

[6] M. Grunberg-Manago, personal communication.

with most extracts, Sm was stimulatory with certain extracts whose protein-synthesizing activity was low.[3]

When aminoglycoside stimulation of "correct" incorporation was studied kinetically, prolongation of the period of linear incorporation was observed with both poly A and poly C. This finding suggests that the aminoglycosides may retard messenger decay (perhaps by maintaining the nuclease-resistant polyribosome complex for a longer time), or may allow release of "stuck" polypeptide chains, leading to further rounds of synthesis. With poly C, the drug also increased the rate of proline incorporation.

This effect on rate of incorporation might be due to degeneracy of the code, which would allow the use, by misreading, of an alternative species of sRNA for the same amino acid. In addition, distortion of the messenger-sRNA interaction by the drug might allow a less precise fit of the "correct" sRNA, and hence an increased frequency of effective "hits" with the ribosome. This kinetic effect, however, would not appear to explain the observation that aminoglycosides increase the binding of lysyl-sRNA to ribosomes in the presence of poly A or of ApApA (4).

While the attachment of different species of sRNA to the ribosome could have additive kinetics at low sRNA concentrations, at higher concentrations competitive kinetics would be expected, akin to competitive inhibition of an enzyme by analogs of the substrate. The ribosomes would presumably approach saturation, and the reading by "incorrect" sRNA's would occur at the expense of the "correct" reading. It is therefore possible to understand how a drug that stimulates the "correct" amino acid incorporation with an unsupplemented extract can become inhibitory at higher sRNA concentrations (see Fig. 2). Indeed, further support for competition is provided by evidence that inhibition of "correct" reading and stimulation of "incorrect" reading result from the same interaction of drug and ribosome: the poly U system shows a different pattern of response to changes in concentration of three drugs (Sm, Nm, and Hm), and in the characteristic concentration range of each drug the inhibition of phenylalanine incorporation parallels the stimulation of serine or isoleucine incorporation (to be published).

Marked effects of sRNA concentration, also interpreted in terms of competition, have been reported for misreading induced by changes in pH and in Mg^{++} (14) concentration, and by streptomycin (4). The latter work demonstrated that at low concentrations of mixed aminoacyl-sRNA's, in the presence of poly U, isoleucyl-sRNA would bind efficiently to ribosomes, but at higher concentrations the isoleucyl-sRNA was displaced. Furthermore, Sm prevented this displacement. It is apparent, from the results presented here, that with increasing sRNA concentration the inhibition by the drug increases, while the relative misreading decreases. It therefore seems important to note that even the highest levels of sRNA used here would be considerably lower than those found in the cell (6). Hence the levels of misreading seen thus far *in vitro* probably exaggerate those occurring in the cell. The early and irreversible inhibition of protein synthesis in the streptomycin-treated cell may represent an extrapolation (to high sRNA concentration) of the inhibition seen *in vitro* (whatever the mechanisms responsible) or may involve additional mechanisms.

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